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Volume 9 January 15, 1959 No. 1

ON THE PRINCIPLES OF CLASSIFICATION AND NOMENCLATURE OF ENTEROBACTERIACEAE

F. Kauffmann

From Statens Seruminstitut, Copenhagen
(Director J. Ørskov, M.D.)

The following abbreviated definitions are common in biology: "each distinct kind of bacterium is called a species" and "a genus is a group of related species." Therefore, it is justified to call the Salmonella group a genus and the Salmonella sero-types species as all the established sero-types according to the Kauffmann-White schema are distinct kinds of bacteria. The definition of the "Salmonella group" given by P. Bruce White in 1929 begins with the words: "A large genus of serologically related, Gram-negative and non-sporing, bacilli" and ends with the sentence: "All the known species are pathogenic for man, animals, or both."

In his 1st monograph together with W.G. Savage "An investigation of the Salmonella group, with special reference to food poisoning" P. Bruce White has said of the absorption types (page 14): "They are elementary species par excellence." This was repeated in his 2nd monograph "Further studies of the Salmonella group" in the following sentence (page 135): "There is every reason to regard these as elementary species, the products of definite phylogenetic events, there is nothing to suggest transmutability among the known types." Further in his "Remarks on the Nomenclature and Classification of Bacteria" in 1936 during the 2nd Congress of Microbiology in London, he wrote the following:

> 6. The level and nature of the bacterial genus and species is a matter calling for consideration.
>
> It is reasonably clear that such an accepted genus as Salmonella, presenting types honoured with specific names, is actually of the same order as pneumococcus, treated usually as a single species enclosing numbered subtypes or varieties. Convenience, history, and the

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characters of the organisms themselves may favour the difference of treatment but the fact remains that the Salmonella type and the Pneumococcus type are of equivalent natural status and are elementary species in the sense of de Vries, and that Salmonella and Pneumococcus are natural genera roughly defined by their R variants and by their basal proteins and polysaccharides.

The Committee might usefully reach a decision as to whether the taxonomic species should be established at the level of the elementary species or whether its size in different cases should continue to be dictated by convenience.

According to the recommendations of P. Bruce White, the designations genus for the whole group and species for the sero-types were adopted by the Salmonella Subcommittee in the first publication on "The genus Salmonella Lignières, 1900," published in 1934. According to this proposal, the Nomenclature Committee in 1936 "approved the definition of the genus Salmonella Lignières as set out in the report and accepted the type-species as Salmonella cholerae-suis (Smith)."

This was approved again by the 4th International Congress of Microbiology in Copenhagen 1947 when an emended Salmonella definition was given. This definition too begins with the words: "A large genus of serologically related, Gram-negative and non-sporing bacilli" and ends with the sentence of P. Bruce White: "All the known species are pathogenic for man, animals, or both."

A change occurred in the Report of the 5th International Congress of Microbiology in Rio de Janeiro 1950 in which the words genera and species are substituted by the words "group" and "types." This was stressed in the Report to the 6th International Congress of Microbiology in Rome 1953 in the following way:

Since differences of opinion may arise in regard to nomenclature, and since the majority of the members of the Subcommittee prefers the terms "groups" and "types" for "genera" and "species", insofar as possible only common names are used throughout the report.

This attitude was adopted by Kauffmann in his book "Enterobacteriaceae" and it was emphasized that the term species was applied only to biochemically defined subdivisions of genera: "Therefore, the sero-types of Salmonella, Arizona, Escherichia etc. are not to be taken as species."

Today, the author wants to eliminate this sentence as he regards only the tribes and genera as biochemically defined subdivisions of the family while the species are the sero-types, according to the definition of P. Bruce White and of the Salmonella Subcommittee. When Salmonella choleraesuis was accepted in 1936 as the type-species, all other sero-types were regarded as species too. These species are in reality large groups which consist of a lot of fermentative types and of phage-types. Therefore, these fermentative types and the phage-types should be regarded as subspecific entities, while the sero-types are species.

What is valid for the genus Salmonella is valid for all the other genera like Escherichia in which the sero-types should be regarded as species too. Consequently, all Shigella sero-types are regarded as species, while the sub-groups A, B and C (= Shigella dysenteriae, Shigella flexneri and Shigella boydii) are no species, but sub-genera, i.e., biochemically defined sub-groups. Shigella sonnei is a species as it is a distinct sero-type.

In the genera Klebsiella and Cloaca the sero-types are the species, while biochemically defined sub-groups are no species, but sub-genera.

The same is valid for the genera Arizona, Citrobacter, Serratia and Providencia.

The species of the genus Proteus are the sero-types according to Kauffmann and Perch, while Morganella, Rettgerella and Providencia are not species, but biochemically defined genera.

Table 1. Family Enterobacteriaceae

Tribes	Genera	Type-Species
I. Salmonelleae	1. Salmonella	Salmonella cholerae-suis
	2. Arizona	Arizona 1,2:1,2,5
II. Escherichieae	3. Escherichia	Escherichia coli (1:1:7)
	4. Shigella	Shigella dysenteriae (A1)
III. Klebsielleae	5. Citrobacter	Citrobacter freundii (1:1,2)
	6. Klebsiella	Klebsiella pneumoniae (1:1)
	7. Cloaca	Cloaca.....
	8. Hafnia	Hafnia.....
	9. Erwinia	Erwinia.....
	10. Serratia	Serratia.....
IV. Proteae	11. Proteus	Proteus hauseri (1:1)
	12. Morganella	Morganella.....
	13. Rettgerella	Rettgerella.....
	14. Providencia	Providencia 1:1

In Table 1 a classification and nomenclature of Enterobacteriaceae is given in which the subdivision into 4 tribes is based on a biochemical basis:

Tribes	KCN	Glutamic acid decarboxylase
I. Salmonelleae	-	-
II. Escherichieae	-	+
III. Klebsielleae	+	-
IV. Proteae	+	+

While the genera are biochemically defined (see Kauffmann: "Enterobacteriaceae"), the species are the sero-types. One of these sero-types is regarded as the type-species, e.g., Salmonella cholerae-suis in the genus Salmonella or Arizona 1,2:1,2,5 in the genus Arizona, a special sero-type with the O antigens 1, 2 and the H antigens 1, 2, 5 or Escherichia coli 1:1:7 = a special sero-type with O antigen 1, K antigen 1 and H antigen 7.

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In the International Bacteriological Code of Nomenclature (Jour. Bact. 55:287, 1948) it is suggested in Recommendation 8a (page 293) "that the terms serotype (or serological type), biotype (or physiological type) and morphotype (or morphological type) may appropriately be substituted for type as a designation of a subdivision of a species." While it is correct to designate biotypes and morphotypes as subdivisions of a species, it is wrong to regard the serotypes as subdivisions. Therefore it is requested to change the International Code of Nomenclature and to permit the designation of serotypes as species.

I should like to ask the Judicial Commission for an Opinion.

SUMMARY

Tribes and genera are biochemically defined subdivisions of the family Enterobacteriaceae, while species are serologically defined subdivisions of the genera, they are, according to P. Bruce White, "elementary species par excellence."

To reach an international agreement, it is necessary to accept the above principles and to abandon the conception of the species as a biochemically defined subdivision of the genus.

In contrast to tribes and genera which are biochemically defined groups, the species are the sero-types. These sero-types which are of fundamental importance for rapid diagnosis in routine-work, for epidemiological, genetical and chemical investigations, are the basis for classification and nomenclature.

REFERENCES

Kauffmann, F. 1954. Enterobacteriaceae. 2nd ed. E. Munksgaard, Copenhagen.

Salmonella Subcommittee. 1934. Jour. Hyg. 34:333.

Savage, W.G. and P. Bruce White. 1945. An investigation of the Salmonella group, with special reference to food poisoning. Spec. Rep. Ser. 91. Med. Res. Council, London.

White, P. Bruce. 1926. Further studies of the Salmonella group. Spec. Rep. Ser. 103. Med. Res. Council, London.

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_____. 1937. Remarks on the nomenclature and classification of bacteria. Repts. Proc., Second Internatl. Cong. for Microbiology, London, page 547.

_____. 1937. Zentr. Bakteriol. II, 96:145.

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Volume 9 No. 1 January 15, 1959 pp. 7-8

DEFINITION OF GENERA AND SPECIES OF ENTEROBACTERIACEAE[1]

Request for an Opinion

F. Kauffmann
Chief, International Salmonella and Escherichia Center

To the Judicial Commission:

In the preceding paper by F. Kauffmann: "On the principles of classification and nomenclature of Enterobacteriaceae" which is the basis for this request for an Opinion, the following summary was given:

> Tribes and genera are biochemically defined subdivisions of the family Enterobacteriaceae, while species are serologically defined subdivisions of the genera, they are according to P. Bruce White 'elementary species par excellence.'
>
> To reach an international agreement, it is necessary to accept the above principles and to abandon the conception of the species as a biochemically defined subdivision of the genus.

According to the recommendation of P. Bruce White, the designations genus for the whole Salmonella group and species for the serotypes were accepted by the Salmonella Subcommittee in the first publication on "The genus Salmonella Lignières, 1900," published in 1934. According to this proposal, the Nomenclature Committee in 1936 "approved the definition of the genus Salmonella Lignières as set out in the report and accepted the type-species as Salmonella choleraesuis (Smith)."

According to Principle 1 of the Bacteriological Code "the essential points in nomenclature are (1) to aim at fixity of names; (2) to avoid or to reject the use of forms and names which may cause error or ambiguity or throw science into confusion."

---

[1]From Statens Seruminstitut, Copenhagen (Director: J. Ørskov, M.D.)

It is evident that there will be no confusion if we continue to call the Salmonella group a genus and the serotypes species. On the other side, if the taxonomic species is not established at the level of the elementary species, i.e., of the sero-types, but on a higher level, e.g., the genus level, confusion would be the result as all accepted names should be changed.

If the genus Salmonella would be regarded as species of genus Enterobacter, Salmonella typhi should be called Enterobacter salmonellae var. typhi.

If the serological subgroups A, B, C, D etc. are regarded as species, the name of the species corresponding to O group B would be S. paratyphi B, but S. typhi murium should be called S. paratyphi B var. typhi murium.

These examples will be sufficient to demonstrate the absurdity of such a nomenclature. It is wrong to regard some serotypes as species and others which are on the same level as subspecific entities. The established serotypes are large groups which consist of many fermentative types and phage-types. Therefore, these fermentative types and phage-types are subspecific entities, while the serotypes are species.

What is valid for the genus Salmonella is valid for all the other genera of Enterobacteriaceae in which the serotypes should be regarded as species.

SUMMARY

The Judicial Commission is requested to give an Opinion on the following proposals:

1. The species of Enterobacteriaceae are the established serotypes.
2. The genera are groups of related species.

REFERENCES

Kauffmann, F. 1959. Internatl. Bull. Bact. Nomen. and Tax. 9:

Salmonella Subcommittee. 1934. Jour. Hyg. 34:333.

White, P. Bruce. 1937. Reports of Proc. Second Internatl. Cong. Microbiol. p. 547 and Zbl. Bakt. II, 96:145.

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MINUTES OF THE VIRUS SUBCOMMITTEE OF THE INTERNATIONAL COMMITTEE ON BACTERIOLOGICAL NOMENCLATURE

A meeting of the Virus Subcommittee of the International Nomenclature Committee was held at the Karolinska Institutet, Stockholm, on the 2nd August, 1958.

Members present: C. H. Andrewes (Chairman), G. H. Bergold, L. M. Black, F. M. Burnet, W. A. Clark, S. T. Cowan, S. Gard, G. Henneberg, P. Manigault (alternate to P. Limasset), I. W. Prentice, R. H. Thung.

Also present: J. M. Birkeland, G. Dalldorf, A. B. Sabin, K. M. Smith.

Dr. Black and Dr. Bergold said that they were in favour of using Linnaean binomials. Dr. Black proposed that plant virus workers should be encouraged to group viruses together and to produce monographs on those viruses whose characteristics were already fairly fully known. Sir Macfarlane Burnet and Professor Gard thought that, at present, group names served a useful purpose but that species names were of doubtful value and that Linnaean binomials were inappropriate. The Chairman pointed out that the Rome proposal to use non-Linnaean names was a compromise between opposing views. Replies to the questionnaire that he had circulated showed that a majority of virus workers in all fields favoured a common system of nomenclature for all viruses. The Rome decisions should not be reversed without full consideration; and the use of binomials in Linnaean form might give rise to nomenclatural problems of validity and priority in other fields of microbiology. Dr. Sabin suggested that Dr. Black's proposals did not in any way contradict the Rome decisions except as regards the use of Linnaean names. It was not obligatory to use the "virus" suffix system if plant virologists could produce an alternative means of indicating that a binomial system did not purport to be Linnaean. Dr. Manigault thought that many plant viruses were too little known to permit of a complete classification. Names of authorities could be appended to binomials but this was not obligatory.

The Committee agreed that the principles of the Rome proposals should not be abandoned and suggested that the Plant Virus subcommittee should consider alternative systems of non-Linnaean names in binomial form.

The Chairman explained the potential duplication of effort in the plant virus nomenclature field caused by the existence of a committee set up by the Botanical Congress. The Committee agreed that Dr. Andrewes should ask Dr. Ramsbottom to raise the question at the next Botanical Congress with a view to transferring responsibility to this Committee.

It was agreed to adjourn the meeting to allow further discussion in the Subcommittees and to reassemble on August 7 at the Technical High School.

Meeting of Animal Virus Group: August 2

Members present: Andrewes, Burnet, Clark, Cowan, Henneberg.

Also present: Dalldorf, Sabin.

The animal virologists then met separately and concluded as follows:

1. As regards animal viruses, the subcommittee's important function at present was to define "groups," taxa above groups could not now be considered and bestowal of specific (group members) names was not urgent.

2. Herpes virus group. Dr. Sabin's study group should not be pressed to publish a description, pending reinvestigation of relations of pseudorabies to herpes simplex and B viruses.

3. Enterovirus group. "Enterovirus" appeared to be more suitable as a group-name than "poliovirus"; this name should therefore be dropped as one equivalent in status to Myxovirus, etc. The Chairman should write to Dr. J. Melnick, Chairman of an American enterovirus committee, telling him that the international group recognized the status of Enterovirus as an official group name and asking his committee to function as the Enterovirus study group under the International Committee, co-opting for the purpose about 3 members from outside the U.S.A., to be selected by them and approved by the Chairman of the Virus Subcommittee. It was recognized that for operational purposes it was convenient to have this difficult group discussed by members who could readily meet in one country but it seemed important that the non-American members should be consulted as freely as was practicable. The study-group should be asked to define the Enterovirus group more precisely than had yet been done, laying emphasis on such

matters as size, nucleic acid composition, resistance to ether and other agents, ability to pack in crystal-like aggregates—rather than on host-range and pathogenic effects produced. They should consider also the appropriateness of bestowing specific names at this stage. Dr. Sabin promised to discuss the matter with Dr. Melnick in Lisbon. The question of designating reference centres for Enteroviruses in the U.S.A. and elsewhere should be reviewed.

4. Adenovirus group. It was agreed to recognize this as a group name. At present it was perhaps best considered as containing but one member, with serotypes. It was, therefore, not urgent to propose a specific name at present. The Chairman was asked to appoint a study-group in consultation with Dr. R. Huebner.

5. Myxovirus group. Several strong candidates for inclusion had appeared since 1953 and it seemed appropriate that the Myxovirus study-group should review them and publish a supplementary list of myxoviruses. For this purpose the Chairman should review the composition of the study-group; the names of Professor Zhdanov and Dr. Chanock were suggested as suitable for inclusion. Again, the question of constituting one or more reference laboratories should be considered.

6. Arborviruses. This name was considered as having wider application than a group-name and therefore should not be authenticated as such. The meeting did not condemn its use as a shortened form of arthropod-borne virus since this was not in their province. The Chairman was to ask the existing study-group to consider proposing group-names for definite separable groups of arthropod-borne viruses, perhaps Casals' A, B, C groups as a beginning: proposal of specific names should not be considered as urgent. The group should be defined taking into account size, resistance to ether and desoxycholate, pathogenicity for vertebrates, relation to arthropod vectors and antigenic characters. Allowance should be made for variant clones which might lack some one important character.

Meetings of Plant Virus Group

(i) On August 2 at the Technical High School, Stockholm.
Members present: Thung (in the chair), Black, Manigault, Prentice.

(ii) On August 6 at Uppsala.

Members present: Thung (in the chair), Bennett, Black, Harrison (alternate for Bawden), Prentice.

Also present: Lihnell, Maramorosch, K.M. Smith.

The group had considered the list of names proposed by a committee set up by the Botanical Congress and had decided not to recommend their use. It was thought that the binomial form of the names might be misinterpreted and taken to imply that the viruses had been classified. Two motions were passed nem. con. at the meeting on the 6th August:

1. That the Plant Virus Subcommittee recommends that plant virologists attempt groupings of plant viruses on the basis of morphology, serology or any other test which there is reason to believe indicates relationships.

2. That if Latinized names are given to such groups of viruses they should be formed on the "Myxovirus" model in accordance with the proposals agreed at the Rome Congress.

- - - - - - - -

The main Subcommittee met at the Technical High School, Stockholm, on August 7, 1958.

Members present: Andrewes (Chairman), Bergold, Burnet, Henneberg, Manigault, Prentice.

Also present: Dalldorf, Sabin, Steinhaus.

The Subcommittee approved the report of the Plant Virus committee and, with minor amendments, that of the Animal Virus group (as circulated).

Dr. Bergold presented his resignation from Chairmanship of the Insect Virus group and Dr. Steinhaus agreed to succeed him in this capacity.

C.H. Andrewes
Chairman

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AGENDA FOR ROUND TABLE EVALUATION OF CRITERIA FOR TAXONOMY*

D. Gottlieb

Department of Plant Pathology,
Urbana, Illinois, U.S.A.

A. Difficulty with many of the current procedures

1. A large number of criteria have been used but were carried on in a different manner by investigators over the past years.
2. Difficulty of reproducing some media.
3. Limits for criteria are not sharp enough. Therefore, interpretation of the test varies.
4. Absence of many type cultures or neotypes.
5. Changes in type cultures.
6. Variability of cultures.

   a. History of handling
   b. Genetic variability
   c. Cultural variability

7. No uniformity among investigators for types and numbers of differences in creating new species (Lumpers vs. Splitters)

B. Evaluation of criteria

1. Standards for criteria

   Are they sufficiently clear so that the results can be interpreted uniformly by investigators in laboratories all over the world?

   Are the constituents of the media widely available?

   Can the tests be standardized?

   Are the reactions stable enough so that they do not change with slight variations in environmental and cultural conditions or variations of the microorganism?

2. Discussions of criteria

Morphological characteristics
- Colony
- Mycelia
- Sporophore structure
- Method of spore formation
- Spore morphology

Color characteristics
- Soluble pigments
- Color of mycelium
- Spore and aerial mycelium

Growth response to temperature

Metabolic characteristics
- Hydrogen sulfide production
- Reduction of nitrates
- Gelatin liquefaction
- Milk peptonization
- Carbohydrate utilization
- Nitrogen utilization
- Production of antibiotics
- Sensitivity to antibiotics

Serology

Phage specificity

C. Speciation

D. Nomenclature

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*Presented at Round Table Conference on Streptomyces, August 4-5, 1958, Stockholm, Sweden, 7th. International Congress of Microbiology.

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PROTOCOL OF THE "ROUND TABLE CONFERENCE ON STREPTOMYCES" ON THE OCCASION OF THE 7TH INTERNATIONAL CONGRESS OF MICROBIOLOGY, STOCKHOLM, AUGUST 4 and 5, 1958

List of Participants

1. Prof. Dr. E. Baldacci, Milano, Italy. 2. Prof. Dr. R.E. Buchanan, Ames, Iowa, USA. 3. Prof. Dr. R. Ciferri, Pavia, Italy. 4. Dr. R. Despois, Vitry-sur-Seine, France. 5. Prof. Dr. L. Ettlinger, Zürich, Switzerland. 6. Dr. W. Frommer, Wuppertal-Elberfeld, Germany. 7. Prof. Dr. G. Giolitti, Milano, Italy. 8. Prof. Dr. D. Gottlieb, Urbana, Illinois, USA (Chairman). 9. Dr. E. Grossbard, Haidenhead, Berkshire, Great Britain. 10. Prof. Dr. J. Horvath, Gödollo, Hungary. 11. Dr. R. Hütter, Zürich, Switzerland. 12. Dr. E. Katz, New Brunswick, New Jersey, USA. 13. Prof. Dr. N. A. Krasilnikov, Moscow, USSR. 14. Dr. E. Küster, Braunschweig, Germany (Convener and Secretary). 15. Dr. H. J. Kutzner, Braunschweig, Germany. 16. Prof. Dr. J. Malek, Praha, Czechoslovakia. 17. Dr. N. Pfennig, Göttingen, Germany. 18. Dr. S. Pinnert, Vitry-sur-Seine, France. 19. Dr. A. Sanchez-Marroquin, Mexico City, Mexico. 20. Dr. I. Scotti, Milano, Italy. 21. Dr. L. Silvestri, Milano, Italy. 22. Dr. P. Sneath, London, Great Britain. 23. Prof. Dr. Z. Tešić, Beograd, Yugoslavia. 24. Dr. V. Treccani, Milano, Italy. 25. Dr. G. A. de Vries, Baarn, Netherlands. 26. Dr. K.H. Wallhäusser, Ffm.-Höchst, Germany. 27. Prof. Dr. M. Welsch, Liège, Belgium. 28. Dr. L. Zeller-Celso, Milano, Italy.

The session was opened on August 4, at 2:15 p.m. by the chairman of the conference, Prof. Dr. D. Gottlieb. A short introductory report was made by the secretary (Küster) on the present status of the classification of streptomycetes (See Appendix 1). The individual participants then expressed their basic concepts of this taxonomic problem. Most of the contributions are in manuscript form. Some were already distributed with the letter of the 13th of June or during the proceedings.

Appended to this Protocol are copies of the papers presented (Küster, Hütter, Baldacci, Tešić, Krassilnikov, Welsch, Wallhäusser, and Kutzner).

Horvath referred to the great variability among streptomycetes. This he attributed at least partially to heterokaryosis. This phenomenon consists in the presence of various nuclei of diverse origin in a common mycelium. In the formation of spores, each conidium receives one only of the various nuclei, so that the colonies derived from these conidia exhibit properties differing among themselves as well as from the parent culture.

Frommer observed that a certain characteristic may be constant within one group but variable in another; this results in an inability consistently to classify a given characteristic as strictly constant or variable. Furthermore, he suggested the examination of the color of the substratum mycelia and of the soluble pigments he supplemented by use of simple chemical tests (solubility in different solvents, color change with change in pH, etc.).

Pfennig considered it important from the standpoint of ecological investigations to use a classification based on morphology. After all, the goal of such studies is ability to classify very many cultures on the basis of simply determined characteristics and to place them rapidly in the larger groups.

Despois noted as a serious defect that, in the newer systematic work, essentially only organisms capable of producing antibiotics are considered. The greater problem in systematic work with the streptomycetes is, in his opinion, careful definition of species. He sees further difficulties due to the absence of designated type cultures of species described earlier; this lack makes almost impossible the identification of new isolations with them. Designation of neotypes and their adequate description would eliminate much of this deficiency. His further observations concerned the standardization of experimental conditions and the description of the characteristics. The morphology and the color of the aerial mycelium he considers the most important properties for characterization.

Krassilnikov supplemented his previously submitted and distributed comments, which may be summarized as follows: He views the definition of the species as the greatest problem. As a result of the broadening of our knowledge, species which were once considered uniform are now divided into several species. For example, the old species Actinomyces globisporus becomes as a result a group of 10 species which

differ from one another by the formation of different antibiotics, for example:

Actinomyces streptomicini	= Streptomyces griseus Waksman : streptomycin
A. levoris	: levorin
A. toxicus	: strongly toxic antibiotic with a toxicity that is 10 times greater than that of KCN
A. bacillaris	:

In this group the differentiation of the species is based primarily on the production of different antibiotics.

Similar groups are represented by Actinomyces albus (now divided into 12 to 13 species) and A. coelicolor characterized by the formation of several pigments and again divided into three subordinate groups:

a) 6 species: spiral
b) 2 species: straight
} blue anthocyanin pigment, pH dependent
c) blue pigment, not pH dependent

In all Krassilnikov differentiates ten species-groups.

Reference was made to the phenomenon that the organisms which produce antibiotics are not usually influenced by their own products. It is thereby often possible to identify producers of antibiotics by testing one against the other. This rule, however, is not valid in every case because there are some species which inhibit themselves. Krassilnikov also considers important the cystomary morphological criteria for the characterization of the species.

Gottlieb reported on a meeting of the "Committee on Taxonomy of the Actinomycetes" of the Society of American Bacteriologists. During the meeting, which took place in May, 1958, problems similar to these being considered here were discussed. At 11 different institutes in the United States, characteristics suitable for use in a classification are to be worked out in a common study under specified experimental conditions. If the results of the test of a character are in agreement at all the institutes participating, this specific character will be considered suitable for characterization.

For the further discussion the separate items of the proposed agenda were treated in the order suggested by Gottlieb and with special consideration of the points A_2, B_2, and C.

On A_2: Difficulty of reproducing some media. Standardization difficulties are often encountered in the production of complex culture media. This seems, however, to be less applicable to culture media whose components have not experienced industrial reconditioning. This was shown to be true in the use of potato or oatmeal agar in various laboratories, as well as by use of potatoes of various types and origins (Baldacci, Küster). Media whose names indicate a similar composition, but whose components have experienced dissimilar treatment can naturally not be compared with one another. For example, several laboratories reported that Difco-potato-agar, compared with potato-agar, is (according to Baldacci) a poor substratum for streptomycetes. Wallhäusser stated that in the preparation of synthetic media the source of the chemical ingredients must be considered because of the variable proportions of trace elements and similar substances. According to Scotti, the age of a medium at the time of inoculation may also exercise a certain influence on the development of cultures. In choosing the media for streptomycetes, one must differentiate between substrata for the maintenance of cultures and those used exclusively for characterization. Media for maintenance must guarantee, above all, satisfactory development of aerial mycelia (Ettlinger).

On B_2: Discussion of criteria.

1. Morphological characteristics

a) Colony: Consideration of the general morphology of the colony is desirable in characterization. For strains that do not form aerial mycelia on any medium, the nature of the substrata mycelium should be appraised.

b) Mycelia: As a result of a preliminary discussion as to the designation of the several kinds of mycelia; the following definitions were agreed upon:

Substratum mycelium:	the totality of the hyphae growing in the agar or solution
Aerial mycelium:	the totality of the mycelium parts which rise above the surface of the substratum

Vegetative mycelium: the totality of the mycelium not dividing into spores (substratum mycelium + sterile parts of the aerial mycelium)

Sporophores: the parts of the mycelium dividing into spores

In general the morphology of the substratum mycelium does not play an important part in the description of the strain.

c) Sporophore structure: There was complete agreement on the importance of the description of the morphology of the aerial mycelium. The division into seven morphological types, which was recently (1958) suggested by T. G. Pridham and associates, was discussed and recommended for general application. Baldacci suggested the following minor changes, among others:

	Pridham	Baldacci
Sectio rectus flexibilis	straight flexuous fascicled	straight flexuous
Sectio retinaculum-apertum	primitive spirals open loops	open loops
Sectio spira	open spirals closed spirals	spirals

Kutzner showed some photomicrographs and discussed the way in which he described the aerial mycelium. Special emphasis was laid upon the arrangement of the spirals on the aerial mycelium, as well as their form and the number of coils. The constancy of the direction of rotation (sinistro, dextro) which he advocated was not approved by all and was therefore considered to be unessential for characterization. It was suggested that the description of the aerial mycelium be supplemented by a photograph. Uniformity of spiral morphology accompanied homogeneous development of aerial mycelia on various media, but only when these media favor the formation of aerial mycelia: for example, Frommer found the same aerial mycelium morphology on five different culture media.

d) Method of spore formation: A differentiation is made between segmentation and fragmentation as methods of spore formation. Within the genus Streptomyces fragmentation is generally assumed, while segmentation is typical for Nocardia. According to Krassilnikov, some Streptomycetes do, however, show segmentation; in this respect, they are similar to Nocardiae. The method of spore formation should be used more for a differentiation of these genera than for species determination.

e) Spore morphology: The observation of the spores by use of a light or a phase-contrast microscope (Giolitti, Ciferri, Küster) allows too limited a recognition of morphological differences that might be used as reference criteria in the identification of species. Unquestionable morphological differences became apparent for the first time with the use of the electron microscope. The fine structure of the spore surface which is recognizable here (smooth, spinous, hairy) is viewed as a very constant characteristic and therefore as an important property for characterization (Hütter, Baldacci). As in the judgment of the morphology of the aerial mycelium, a culture medium should be used for this criterion which guarantees a good formation of aerial mycelia and spores, for, on an unsuitable medium the characteristic appendages, which are otherwise apparent, may be absent (Küster). The preparation should be made by the print-process, as the spores may lose their appendages by suspension (Küster).

A description of the spores as shown by the electronic microscope is considered essential for an adequate species description. The use of this characterictic in a key for determination, as Ettlinger and Hütter suggested, is probably justified, but the use of such characters in a key makes difficulties for the student, however, because electron microscopes are not available everywhere. This deficiency may be overcome by an exchange of strains.

2. Color characteristics

All three components of a streptomycete culture, aerial mycelium, substratum mycelium and medium (substratum), may develop colors which can be used in characterization.

a) Color of the aerial mycelium: The color of the aerial mycelium was defined as the color of the mature aerial mycelium on a culture medium which is optimal for

the production of aerial mycelium and of spores. This color is generally regarded as constant and its description essential for characterization. Baldacci suggested that in addition to a description of the mature stage, there be evaluation of intermediate stages. On the basis of an observation on the color change of the aerial mycelium of Streptomyces griseus subjected to iodine fumes, de Vries pointed to the possibility that the color of the aerial mycelium may be characterized by chemical reactions.

b) Color of the substratum mycelium: This color evaluation was considered less important for characterization, since it is more variable than the color of the aerial mycelium and, in most cases, is not characteristic. For those strains that show a characteristic substratum-mycelium-color, e.g., red, blue, violet, this color should be recorded. In such cases, the dependency of the coloring on the composition and the pH value of the medium should be especially noted (Frommer, Giolitti).

c) Color of the medium: The color of the medium, also commonly designated as soluble pigment, is with a few exceptions, not important, since it is either uncharacteristic or, when strikingly colored, it has the same color as the underside of the substratum mycelium, though less intense. Moreover, the color of the medium is observed to be more variable than that of the substratum mycelium. After several transfers, the pigmentation of the medium may frequently be lost, but may be regenerated by a single passage through soil (Wallhäusser). It must be expressly pointed out that the coloring of the medium, here under discussion, does not include the melanoid pigmentation of culture media containing peptone. In the discussion on color characteristics, it was repeatedly expressed that a distinction must be made between "pigment" and "color." Krassilnikov defined these terms as follows:

Pigment = the chemically uniform metabolic product which can be extracted and is not liable to change by external factors, e.g., 3 types of pigment in Streptomyces violaceus: an anthocyanin, a carotinoid and a lipochrome.

Color = subject to influence by external factors in the culture (trace elements, pH value etc.)

The color of the mature aerial mycelium (spores en masse) in all cases should definitely be described. In some cases, the color of the substratum mycelium or, occasionally, the color of the medium may be noted; for these the specific medium, pH changes, and similar data must be described.

3. Growth response to temperature

Most of the streptomycetes are mesophilic and have very similar temperature requirements, only a few deviate and are thermophilic. Only in the cases in which the optimal temperature lies substantially above that of the mesophilic species should the temperature requirement be used as a characteristic in the differentiation of the species.

4. Metabolic characteristics

a) Hydrogen sulfide production: testing for the formation of H_2S was not considered necessary.

b) Reduction of nitrates: Only limited experience with this test was available, but as far as the test applied, it represented a good characteristic. It is recommended.

c) Gelatin liquefaction: Differences are noted solely in the rate of the liquefaction. This test is applicable in the description, when the length of time for liquefaction to occur is recorded.

d) Milk peptonization: In light of the experience of several participants, this test seems to be strongly dependent on the external conditions of the experiment. For example, de Vries observed a distinct influence of the temperature and length of time of sterilization, as well as of the origin of the milk on decrease in peptonization. This result may also depend on the size of the inoculum (Hütter). A further difficulty is present in the variability in the description of the results: for example, in one case only a thick flocculation may be designated as coagulation, while in another case a "more bilious" condition is also designated as coagulation (de Vries). Because of the difficulties in carrying out such a test with fluid milk, de Vries and Wallhäusser suggested the use of milk agar or, under some circumstances, casein agar. Ciferri called attention to a method of Gorini by which the old agar cultures are covered with a layer of milk; in comparison to the customary milk test in tubes, coagulation is observed much more frequently.

Despite the difficulties indicated, the milk test is given a certain value, although its application presupposes an exact adherence to standardized experimental conditions. All participants who occupy themselves especially with this test are asked to communicate their experiences to Prof. Gottlieb.

e) Carbohydrate utilization: The lively discussion on this test can be summarized as follows:

1. This test is viewed by many participants as useful (Wallhäusser, Sanchez-Marroquin, Pfennig, Pinnert, de Vries, among others) but it must be limited to a few carbohydrates, since many do not yield results which are reproducible (Pfennig, Sanchez-Marroquin). When others are tested they reveal few differences among most of the streptomycetes.

2. Hütter and Ciferri, on the basis of their studies, reject this test completely; Hütter, for example, found that single spore cultures of a single strain may possess different C-source spectra, The observations of Pinnert are somewhat similar. She found that, following a reaction generally regarded as negative, individual positive colonies appear which she designates as mutants. Difficulties in the exploitation of this test are seen by Giolitti in the fact that some strains develop on the basal medium without the addition of carbohydrates.

3. It was noted concerning the methodology of this test that it should be carried out either on agar or in a fluid medium in a submerged culture. Cultures floating on solutions lead to unsatisfactory results (Küster). Wallhäusser utilized successfully the submerged culture; he found that the amount of mycelium can be determined quantitatively after centrifugation.

Despite the critical comments, the use of the C-test is recommended for the characterization of a strain. Pinnert is asked to send to Küster an exact description of the method of this test which she used. Likewise, all participants are asked to communicate their experiences with this test, particularly with reference to the selection of C-sources, to Küster.

f) Nitrogen utilization: As in the test for C-source, the utilization of various N-sources by strains was evaluated very differently by the participants. Pinnert reached good results with such tests, while Ciferri considers the N-source spectrum uncharacteristic. Too little experience is available to arrive at a final judgment.

g) Production of antibiotics: The formation of antibiotics is rejected as a criterion for the characterization of a species for the following reasons:

1. This property is not considered constant, because a loss of antibiotic formation can frequently be established under laboratory conditions.

2. There are numerous species of streptomycetes which contain inactive strains, as well as strains that produce antibiotics.

3. In several cases more than one antibiotic was formed by one strain.

4. The same antibiotic can be formed by different species. Kutzner established that, in many cases, strains which were very similar to their morphological and cultural characteristics on different culture media also exhibited a corresponding antibiotic spectrum with five different test organisms. On the basis of this observation, he comes to the conclusion that reference can be made to the antibiotic spectrum for classification of subgroups. The question remains unanswered, however, to what extent the spectrum can be brought about by the same antibiotics or, as the case may be, by the same mixture of antibiotics. It once more turns out that the formation of antibiotics or, more exactly, the antibiotic spectrum, is useful only for a description of a strain and not for the definition of a species.

h) Sensitivity to antibiotics: Only very limited experience with this test is available. Krassilnikov uses it for diagnostic purposes.

5. Serology
Those present had had no experience in this method of investigation.

6. Phage Specificity
Kutzner reported on his investigation with five different phages in his group I (corresponding approximately to the griseus group). Two phages proved to be quite specific, that is: only a few sub-groups of streptomycetes are sensitive; the remaining three, among which was the phage specific for Streptomyces griseus, had a very wide spectrum. This differing specificity of the actinophages must be considered in evaluating their usefulness in diagnosis of streptomycetes. Baldacci has worked with phages that were specific only for the griseus group but for none of his other series.

On C: Speciation
Baldacci presented in the following diagram his conception of the characteristics which he suggests for a species and infrasubspecific division:

Species	sporophore structure
	color vegetative mycelium
	color aerial mycelium
	spore morphology (electron microscope)
Infraspecific	metabolic characteristics
	soluble pigments
	antibiotics (?)

Sneath explained his ideas on speciation and called attention to his account already published (Jour. Gen. Microbiol. 17: 184, 201, 1957). Gottlieb suggested that the various laboratories exchange strains in order to establish through use of uniform record cards the stable criteria suitable for characterization. A plan was developed to carry out a common experiment somewhat similar to the study being made by a taxonomic committee of the Society of American Bacteriologists. Participating in this experiment will be 14 individuals using the same strains (from Baldacci's collection) under standardized experimental conditions, including observation and judgment as to the morphology of the aerial mycelium

and spores and the color of the aerial and substratum mycelia as well as the intensity of the melanoid pigment. In this way, the reproducibility of these characteristics should be co-ordinated. The specific arrangement for the experiment will be worked out and forwarded by Küster, who will then assume the task of the final evaluation after conclusion of the experiment.

Earlier in the session Buchanan had suggested the possibility that it might be desirable and advantageous for the conference to seek recognition as a continuing taxonomic subcommittee of the International Committee on Bacteriological Nomenclature under the auspices of the International Association of Microbiological Societies. The suggestion met with the approval of all participants. It was formally resolved to form a "Subcommittee on Streptomycetes" within the International Committee on Bacteriological Nomenclature. Gottlieb was elected chairman, and Küster secretary of the Subcommittee. After 3 half-day sessions, Gottlieb closed the meeting on August 8, 1958, at 6 p.m. with a few words.

Dr. E. Küster
Secretary

INTERNATIONAL BULLETIN OF BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY
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REMARKS ON THE CLASSIFICATION OF STREPTOMYCES*

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Any single character of a microorganism is likely to vary within more or less wide limits. Therefore (a) a large number of different characters should be used, and (b) more weight should be given to characters that experience has shown to be least variable. The latter will usually be well-chosen morphological features whose presence is governed by the interaction of many different genes.

With only a few of the characters discussed at the Conference have I had personal experience.

a) Antibiotic production: This type of property can have at most a secondary value only, since it is subject to wide quantitative variations and since a given antibiotic can be produced by several completely unrelated organisms.

b) Antibiotic sensitivity: This type of property can be more useful. Admittedly, mutants resistant to any antibiotic can be obtained by proper techniques. However, the appearance of such mutants and their selection under natural conditions (i.e., in the absence of laboratory manipulations) is not likely to occur to any appreciable extent when it must be the result of multiple step mutations, as is usually the case.

In fact, studying Corynebacteria isolated from the human respiratory tract, the frequency-distribution of sensitivity to several antibiotics was found to be different for groups of strains considered, on other grounds, to belong to distinct species (Welsch et Thibaut, Ant. v. Leeuwenhoek, 14:193, 1948; C. R. Soc. Biol. 143:1284, 1949; Thibaut, C.R. Soc. Biol. 146:143, 1952).

*Presented at Round Table Conference on Streptomyces, August 4-5, 1958, Stockholm, Sweden, 7th. International Congress of Microbiology.

c) Production of specific actinophages (lysogeny) and sensitivity to actinophages: Again these properties are subject to wide variation when the organisms are subjected to appropriate laboratory procedures, but the occurrence of such variations under natural conditions is probably much rarer.

A review of the literature shows that many actinophages have a very wide spectrum of activity. They cannot be very useful for identification and classification of species. However, a recent paper (Bradley and Anderson, Science 128:413, 1958) demonstrated by their use the affinity of species placed in two genera, Streptomyces and Nocardia included in two different families.

On the other hand, actinophages with a much more restricted range of activity are also known. A group of phages was shown to act exclusively on strains belonging to the Streptomyces griseus group (Welsch, Corbaz and Ettlinger, Schweiz, Z. Path. Bakt. 20:454, 1957; Welsch et Pinkaers, C.R. Soc. Biol. 151:1283, 1957) as defined by Ettlinger and co-workers (Giorn. Microbiol. 2:91, 1956).

One of the viruses (Welsch, Giorn. Microbiol. 1:339, 1956; Ant. v. Leeuwenhoek, 23:59, 1956) acted, although with quantitative differences, upon all the organisms of the group which were tested (but one which proved to be a lysogenic strain carrying a related prophage) and had no action except in a few cases even when used in a high concentration, on unrelated streptomycetes.

We therefore believe that phage sensitivity and prophage carrying (Welsch, Virology, 2:703, 1956; Bull. Res. Counc. Israel, 7E:141, 1958) are properties likely to help the taxonomist. However, much remains to be done in order to define a proper methodology for their use and we are not now able to propose a technique. Quantitative rather than purely qualitative methods will be necessary. It must also be stressed that most of the actinophages known at present are griseus-phages.

Obviously phages for unrelated actinomycetes should be obtained (Welsch, Minon and Schönfeld, Experientia 11:24, 1955) and studied before a definite opinion about their use can be offered.

d) Genetic interactions between strains: Recent work has clearly shown that heterokaryons and, more rarely, true hybrids can be obtained following the mating of two different mutant strains of an actinomycete. The possibility of mating between organisms considered to be related should strengthen this opinion, and conversely. Perhaps, in the near future, mating will offer a convenient criterion for the practical definition of a species. An eminent French zoologist, once was asked by a friend of mine whether he was right in according species status to a newly described copepod. The answer was: it is a distinct species if it does not cross, or gives only infertile crosses with the other species which it resembles. The criterion was, of course, impractical in the case under consideration since the only representative of the would-be new species had been in alcohol for many years, but techniques could be devised to have this test applied in the case of streptomycetes.

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IDENTIFICATION OF STREPTOMYCETES*

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In the course of our work with streptomycetes we have had many times the problem of identify of an isolation of our own with a known species, or of its recognition with certainty as a new species. The existing systems did not satisfy our needs.

The identification of a streptomycete with the description only is, at least, difficult. Criteria of very different systematic value are to be taken into consideration. There is no clarity as to which characters are important for species delimitation and which are only accessory (ancillary). For a long time production of a soluble pigment was considered to be a criterion of primary importance. Today many authors have replaced it by description of the colour of the vegetative and aerial mycelia and by the morphology of the aerial mycelium.

The system of classification of one author describes a certain strain in quite a different fashion than that of another author. To avoid this source of error we have concluded to rely only on comparison with known strains of culture collections. But this procedure gives rise to still other problems: a) in many cases the original (type) cultures are lost or are not available and, b) often one finds differences between the recorded description of the reference culture and his own observation. Can the differences which are found be regarded as consequences of variability?

As a result of our experiences with many culture collection strains and with our own isolations we have come to the conviction that this frequently cited variability is limited to striking but untrustworthy criteria such as a soluble pigment, antibiotic activity or the colour of the vegetative mycelium.

*Presented at Round Table Conference on Streptomyces, August 4-5, 1958, Stockholm, Sweden, 7th. International Congress of Microbiology.

However there are certain criteria which we consider as constant in the sense that they do not vary during a long observation period in the laboratory, that single spore cultures are homogeneous with respect to these criteria and that these criteria are in general independent of the medium, the culture conditions and the age of the culture.

Distinction based on characters ranked according to their different systematic value have been proposed by many authors, but in all such proposed systems these criteria served only for the characterization of a unit (rank, taxon) between the genus and the species, as Sectio, Series, and Group.

We propose a classification of species which discards all inconstant criteria and those not readily determinable. We regard the species as the lowest systematic unit which can be characterized by constant criteria.

Four groups of characters are suited to such a characterization and differentiation of the species, namely, a) the morphology of the spores under the electron microscope, b) the colour of the aerial mycelium, c) the morphology of the aerial mycelium, d) the ability to form a melanoid pigment in media containing peptone. I present a brief survey of the subdivisions of these four groups of characters.

a) As to the morphology of the spores, I must mention that it is not the form but the outline that is significant. It is important to note whether the spores are smooth or whether they show appendages. Not only the presence of appendages, but also their specific form is constant, as hairs, thorns, or prickles.

b) by the "colour of the aerial mycelium" we mean the colour of the well-developed, mature aerial mycelium, called by some authors "spores en masse." I will not discuss the differences between our colour groups and the colour groups established by other authors. I will merely give a summary of our colour groups. We distinguish

niveus	(snow-white)
griseus	(yellowish-greenish-gray)
azureus	(blue)
cinnamomeus	(pink-cinnamon)
cinereus	(gray)
prasinus	(leek green)

Determination of the colours by use of colour tables did not prove practical, because one cannot indicate the whole range of colour. So we accepted for each colour group certain strains (we took culture collection strains), which showed good formation of aerial mycelium and which showed the certain colour in a typical shade.

c) Our system of defining the morphology of the sporophores is in principle similar to the system of Pridham, Hesseltine and Benedict. But while these authors united several types in the same section we recognized each type as an equivalent unit. Important for the recognition of the type of morphology of the aerial mycelium are the presence or absence of a sterile middle-axis, from which the fertile spore chains branch; the mode of ramification (sympodial-monopodial); the ability or inability to form whorls (it must be mentioned here that we were not able to separate single and double whorls); the ability or inability to form spirals and the kind of spiral (closed-open, regular-irregular).

d) As to the fourth point, the formation of a melanoid pigment, I will only mention that we have a method which allows one to distinguish easily between chromogenic and nonchromogenic types.

Of all the possible combinations we found only a small number realized. So, for example, the colours griseus and cinnamomeus were always associated with smooth spores and the strains with aerial mycelium having colours azureus or prasinus all had spores with hairs or thorns. All the strains with spores with appendages showed spiral formation in the aerial mycelium and finally all strains with aerial mycelium griseus showed sympodially branched tufts with flexuous branches in the aerial mycelium.

As a result of these conclusions we grouped all our strains into species. In our opinion, other criteria as, for instance, soluble pigment, antibiotic activity, colour of the vegetative mycelium, utilization of carbon sources and other physiological criteria can serve only as accessory criteria suitable below the rank of species for the description of a special strain.

In conclusion, I want to say that for us the two most important points for the systematics of the genus Streptomyces seem to be:

a) The fixation (determination) of species with help of type strains, preserved in official type culture collections.

b) The characterization of the species only by employing stable characters and as a consequence the raising of the species level to a reasonable height, so that in future not every strain will be described as a single species.

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SOME PROBLEMS IN THE IDENTIFICATION AND CLASSIFICATION OF SPECIES OF SPIRILLUM
I. EARLIER TAXONOMY OF THE GENUS SPIRILLUM

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Spirillum Ehrenberg, 1830, is one of the oldest bacterial generic names now recognized. The genus was originally characterized by Ehrenberg (1838) as follows:

> CHARACTER: Animal e familia Vibrioniorum, divisione spontanea imperfecta (et obliqua?) in catenam tortuosam s. cochleam rigidum et in cylindriformam extensam abiens.*

Ehrenberg recognized three species in the genus—Spirillum tenue, S. undula, and S. volutans. One species, S. tenue, was described as new, the other two had previously been included by Müller in his genus Vibrio. Ehrenberg distinguished the species on the basis of the diameter of the cells, the type of spiral curvature, and the type of motility. Essentially the same morphological characters were used throughout the next hundred years in the separation of the species. Physiological and biochemical characters have had little application in writing diagnoses of the species of Spirillum. Two reasons for this failure to use physiological characters are: (1) many of the species of the genus were named and described prior to the development of techniques for recognition of physiological differences in bacteria; (2) even more important is the fact that species of Spirillum as studied today show few if any distinctive physiological or biochemical characteristics such as those used in the identification and characterization of many other bacteria.

Species of bacteria are commonly characterized by physiological characters; morphological characters, as a rule, are used only in the higher taxa, such as family and order,

*An animal of the family Vibriones with spontaneous incomplete (and oblique?) division into a crooked chain or a stiff spiral and assuming the form of an extended cylinder.

and are relatively unimportant in distinguishing related genera and species.

Differences in morphology are the only features which distinguish species of the genus Spirillum, the morphological characters first noted by Ehrenberg (1838) are still significant. Additional characters used today (Bergey's Manual, 1957) are the Gram-negative reaction; the possession of polar flagella; a chemoheterotrophic type of metabolism; and the absence of bacteriochlorophyll and sulfur inclusions. While the morphological characters (used by Ehrenberg) as the diameter of the cell and type of spiral curvature are sufficient for the separation of morphological types as species, these characters alone are seldom sufficiently distinctive to provide reliable means for identifying a new isolate with a previously described species. The species of Spirillum are, as a rule, so physiologically inert that the physiological characters commonly used are not distinctive. The introduction by Giesberger (1936) of the techniques of using known carbon compounds as the sole sources of energy in a mineral medium provided physiological characters by which a previously described species could be reidentified, but only when these characters are correlated with a distinctive morphology.

The descriptions of the species of Spirillum Ehrenberg given in Bergey's Manual of Determinative Bacteriology (7th ed. 1957) are quite inadequate for identification. They are composites of the characters assigned to the several species by Giesberger (1936) and those given to the same species by previous students, consequently it is difficult to identify an isolate on the basis of measurements alone. Measurements, unsupported by illustrations of the organism, may lead to errors in identification unless they record the differences in the type of spiral curvature. The uniqueness of a particular morphological type was shown by Williams and Rittenberg (1957) for three different species of Spirillum whose over-all measurements were essentially similar.

Characterization of the species of Spirillum, by comparing descriptions of morphologically similar strains assigned to the same species by various investigators with the physiological characters found for strains assigned the same species by Giesberger (1936), makes exact identification of an isolate of the genus difficult. Ostensibly the species names, Spirillum undula, S. tenue, and S. volutans have

been used by many workers since the time of Ehrenberg (1838), but it is questionable whether the organisms have been correctly identified in most cases. However, at the present time it should be possible to reidentify a previously described species by correlating a unique morphology with the utilization of a specific set of carbon compounds. To identify an isolate of the genus Spirillum it is necessary to include in the comparison both morphological and physiological characters with those of cultures of adequately described species. Such direct comparisons have been sharply limited by the absence of suitable authentic type or standard cultures. If the isolate differs from any available culture of species of Spirillum, the only recourse is to compare its characters with those of other species so inadequately described in the literature, emend the description and designate type or neotype cultures to be deposited in suitable Type Culture Collections.

Much of the literature on the genus Spirillum Ehrenberg appeared prior to 1900, some of it is not readily available. A review shows that spirilla of the same morphologies have been described for a hundred and twenty years under the species names of Spirillum tenue, S. undula, S. volutans, and S. serpens. Some of the problems in the identification of species of Spirillum stem from differences in the morphological and physiological characters ascribed by various investigators to organisms bearing the same species name. The most concise, although not the most exhaustive, sources of information on the genus Spirillum are the records prepared by earlier investigators. Some (not all) of these taxonomic treatises are here considered.* Plates and figures from these earlier publications are reproduced here to illustrate the morphology considered typical of the species by the several authors.

Müller's Taxonomy

The earliest attempt at the classification of microorganisms (animacules) was made by the zoologist, O.F. Müller, in two publications, Vermium terrestrium et fluviatilium, 1773 and Animacula infusoria fluviatilia et marina, 1786.

* For a list of earlier references see Buchanan (1918).

Müller did not distinguish between protozoa and bacteria; in fact this differentiation was not generally recognized until nearly a hundred years later. Müller considered his Vibrio undula to be the "little eel" described by Leeuwenhoek. He says

> Leeuwenhoek points out that it is an animacule whose little body excels in slenderness the end of the tail of the seminal animacule and is ten-hundred-thousand thousand times smaller than a mustard seed. I agree with the given description, and continuing from that point, I gladly quote below from this author: 'Since the animacules contract their little tails when they move in circles and folds, we can rightly conclude that these little tails do not lack tendons, muscles and joints any more than do the tails of the dormouse and the mouse; nor does anyone doubt that these same animacules, which swim in water, are equipped with organs of locomotion just as are larger animals. Furthermore, how much equipment of visceral organs, or how great a lack of them is enclosed in such a small animal?' Likewise, in his Letter (Arcene Naturae 96) he calls it an eel, because when it swims with incredible speed, it bends its body in the manner of an eel.

Müller (1786) included the organisms now recognized as bacteria in two genera, Monas and Vibrio, the spiral forms were assigned to the new genus Vibrio. This genus contained eight species; two were probably not bacteria. Drawings of the six species recognizable as bacteria, are reproduced in Fig. 1. Three of these species can be recognized as spiral forms; Vibrio undula, V. serpens, and V. spirillum. The specific epithets of two of Müller's species are still in use in Spirillum undula and S. serpens. Because of its historical interest, Müller's description of V. serpens is quoted with a rather free translation of the Latin text as follows:

56. VIBRIO SERPENS

A thread-life Vibrio with its windings protracted at an obtuse angle. Table VI. Fig. 7,8.

Especially when extended it is longer than Vibrio

undula by tenfold; it is seen to differ from Vibrio spirillum by its obtuse angles and is differentiated from both of these by its intestine which extends through its entire length, and by its greater size.

It is a gelatinous animacule, extremely slender, showing a linear serpent shape, with even and loose curves, but not in the least manner resembling the fabled river serpent of Norway. When it is extended it is straight, not spiral, it is loosely coiled with serpentine curves.

It is rarely found in river water.

Although it would be difficult at present to identify an isolate of the spiral organisms from the descriptions and drawings given by Müller, the drawings do show that his organisms were morphologically similar to those observed today. The present-day cultures recognized as those of Spirillum undula and S. serpens show only a slight morphological resemblance to their prototypes, Vibrio undula and V. serpens. Nevertheless, Müller's description of V. serpens lists one characteristic which is found in cultures of S. serpens today; namely the tendency of the organisms to appear as straight rods when extended or in rapid motion.

Ehrenberg's Taxonomy

Ehrenberg, like Müller, also included among the protozoa several organisms now placed with the bacteria. Ehrenberg increased the number of genera and species now included in the bacteria, retaining Müller's names Monas and Vibrio for two of his genera. In 1838, in his book Die Infusionsthierchen als volkommene Organismen, Ehrenberg presented a classification of the microorganisms observed by him. He described many species now assigned to bacteria and improved the definitions and diagnoses of the genera as well as the species. In 1829 Ehrenberg had observed a large spiral organism in St. Petersburg which he described in 1830 as Spirillum volutans. Ehrenberg utilized the specific epithet Spirillum of Müller's species, Vibrio spirillum, as the generic name for this organism. In the descriptions of this species in 1838, Ehrenberg discusses his reasons for this change. According to Ehrenberg (1838) Kohler first observed this organism in 1777. He states:

Der Entdecker oder erste Verzeichner dieses Thierchen war 1777 Kohler in Leipzig, dann beschrieb es Hermann in Strassburg aus dem Aufguss von vegetabilischen Kuchen-Abfall und sagt 1784, dass es die Beobachtungen vor 18 Jahren gemacht. Er schickte seine Zeichnung an Müller zur Bestimmung und so erhielt sein Thierchen von Müller den namen Vibrio Undula. Die Zeichnung ist nach zu kleiner Vergrosserung, entworfen, zeigt aber deutlich die Form das Spirillum volutans durch mehrfache Schraubenwindungen. Müller selbst lernte diese Form schon 1782 in einen Aufguss von Sonchus arvensis kennen, hatte aber noch langere Schrauben argetroffen, die ihn bestimmten, jene kurzen von Hermann als V. Undula aufzuzeichen. Auch erst in den spätem Infusionwerk ist bei V. Undula bemarkt, dass es Formen dieser Art mit mehrfachen Spiral windungen gebe.

For these reasons, Ehrenberg (1838) assumed that Hermann's organism and the one which Müller had observed in 1782 were identical with his species, Spirillum volutans. Since the organism observed in 1829 had the many spiral windings depicted by Müller (1786) for Vibrio spirillum, Ehrenberg (1830) used Müller's specific epithet of Spirillum to name the new genus. In the legend fo the various drawings of S. volutans, Ehrenberg (1838) admits the possibility that more than one species may have been observed. Although Ehrenberg (1838) appears to have made a large number of assumptions in regard to his species, S. volutans, he states that Vibrio spirillum is the prototype of S. volutans in the following sentence: "Müller's Spezial-Name ist zum Genus-Namen erhoben worden." In his description of S. undula, Ehrenberg (1838) says that this organism is the same as the one observed by Kohler in 1777. It is possible that Müller and the other authors mentioned by Ehrenberg observed different organisms, it being difficult at the present time to reach a decision from the data given. It should be noted, however, that Müller's description of Vibrio undula, given in the 1786 work, is essentially the same as that published in 1773.

In addition to Spirillum, Ehrenberg named three other genera of spiral organisms. One contained a species, Spirochaeta plicatilis, with flexible cells in contrast to the rigid

spirals of Spirillum. Spirodiscus fulvus, a second species had the shape of a coiled watch spring. Several other species are placed in another genus of rigid spiral forms, Ophidomonas. Ehrenberg (1838) differentiated Spirillum and Ophidomonas on the basis of the presence of a motility organ and complete division in the latter, but not in the former. He also states that the genus Ophidomonas has pigmented species (O. jenensis olive-brown; O. sanguinea blood red). Ehrenberg (1838) did not include Müller's species Vibrio serpens in the genus Spirillum because, as he states in an addendum to the description of the genus, it was obviously a species of the algal genus Oscillatoria.

A portion of Ehrenberg's Plate V (1838), in which the species of Spirillum are illustrated, is reproduced as Fig. 2. A direct comparison of Müller's drawing of Vibrio spirillum (Fig. 1, no. 9) with Ehrenberg's drawing of S. volutans (Fig. 2, no. XIII) leaves little doubt that the two organisms were not the same. Müller (1786) described V. spirillum as being thread-like while the dimensions* given by Ehrenberg (1838) for S. volutans could never be so construed. They agree well with the dimensions of the cells found in the Pringsheim culture of this organism. The cells of the Pringsheim culture often show the tight spiral windings, depicted by Ehrenberg for the organisms observed in 1829 (Fig. 2, no. XIII (2)), particularly when the organisms are growing in a mixed culture containing many protozoa; i.e., conditions similar to those under which Ehrenberg observed his species. Migula (1900) points out that Müller's illustration of V. spirillum resembles Ehrenberg's drawings of Spirochaeta plicatilis more than it does his (Ehrenberg's) drawing of S. volutans. The descriptions given by the two authors for Vibrio undula, V. spirillum (Müller, 1773; 1838) do not permit a decision as to whether Ehrenberg was justified in his revision of nomenclature. The Müller and Ehrenberg illustrations, however, do show the various morphologies of spiral organisms.

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*(Dimensions of S. volutans, Ehrenberg, 1838). Lange der Spirale 1/192 bis 1/48 Linie. Dicke etwa 1/1200 Linie. (A Linie is equivalent to 1/12 of an inch.)

Cohn's Taxonomy

Much of Müller's (1773, 1786) taxonomy of the organisms now included with the bacteria may be dismissed today as of "historical interest" only. Cohn's system of classification (1872) was the beginning of modern bacterial taxonomy (see Bergey's Manual, 1957, p. 6). Of the many systems of classification of the "bacteria," proposed by his predecessors, Cohn considered only those of Ehrenberg (1838) and Dujardin (1841) to have merit and took their publications as the starting point for his own system. Cohn's comments on the difficulties of classifying the bacteria have a familiar ring and are sufficiently relevant to be quoted in translation.

> Whoever studies the pertinent recent literature knows that, to a degree, confusion has arisen in the nomenclature of the bacteria. Almost every investigator, without being concerned with the work of his predecessors, has often designated easily recognized forms quite arbitrarily, assigning to them new names, and the rule of priority, which is the basis of nomenclature of organisms everywhere, is generally disregarded.
>
> In fact, the obstacles encountered in giving these organisms the correct designation and nomenclature are extraordinary. Only Ehrenberg and Dujardin have endeavored to classify entire series of bacteria, to arrange them in relationship (to each other) and to assign them to genera and species, their works, therefore, must serve as the starting point. . .
>
> All of these difficulties make themselves felt when we seek to assign bacteria to their natural genera. The genera of the bacteria have not the same significance as those of higher plants and animals as they are based only on the characteristics of vegetative cell formation and not on reproductive characters. . . . (1872, p.128).

Despite his respect for the classification of Ehrenberg (1838) and Dujardin (1841), Cohn (1872) criticized the diagnostic characters used by these authors to distinguish some of the species. He found it difficult to separate Ehrenberg's Bacterium termo from Vibrio lineola; his Vibrio subtilis from Spirillum tenue; and Vibrio prolifer from Spirillum undula. Concerning these difficulties, Cohn (1872) states:

BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY

> The supposedly good diagnostic characteristics of Bacterium, Vibrio, and Spirillum established by Ehrenberg do not appear so distinctive in their application in reality. To be sure we find rigid, small, rod-like forms which can be placed without hesitation in Bacterium, rigid spirals, which can be placed in Spirillum; then we encounter eel-like forms, darting here and there, which both Ehrenberg and Dujardin designated as Vibriones.
>
> My convictions are that the undulating and spiral forms can be applied collectively to the Vibriones and that the morphologically stable Spirillum is not capable of stretching and bending and therefore has no really sinuous motion; it follows that there is no Vibrio corresponding to Ehrenberg's definition of the genus. It is evident that these genera must be defined anew.

Cohn (1872) divided the bacteria into four groups (Tribes), each containing one or more genera. The sinuous and spiral organisms were placed in two Tribes; Vibrio (char. emend.) was placed in the Tribe Desmobacteria and Spirillum Ehrenberg in the Tribe Spirobacteria. Genus 4, Vibrio, contained two species, Vibrio rugula and V. serpens; genus 5, Spirillum Ehrenberg, contained three species, S. tenue S. undula, and S. volutans. Cohn retained the names of Ehrenberg's (1838) species and reintroduced Müller's (1786) species, Vibrio serpens, since he considered that Ehrenberg was not justified in his rejection of this species. Cohn (1872) described the genus Vibrio (char. emend.) as "characterized by the stable form of the undulating windings of the filaments, which by their rotation produce the appearance of being sinuous and form, therefore, a transition to the spiral bacteria or Spirillum." Cohn also observed that the cells of V. serpens appear as straight rods during motion as earlier noted by Müller (1786).

Cohn separated the genus Spirillum from the genus Vibrio on the basis of "the thicker and tighter soils with a regular form, i.e., stable curves in the filaments" and by the presence of a flagellum in S. volutans. The discovery of a flagellum in S. volutans and not in the other species of Spirillum caused Cohn (1872) to consider transferring this organism to the genus Ophidomonas since Ehrenberg (1838) had described a "snout" on these organisms to be the cause

of motility. S. volutans, however, was colorless and had been so described by Ehrenberg whereas the species of Ophidomonas were described as being colored. Cohn was reluctant to separate S. volutans from the other two species of Spirillum and in discussing a large spiral organism which appeared red in mass, although single cells appeared colorless, Cohn (1875, p. 171) says:

> But a second genus controverts the diagnosis of our species in the bacterial genus Spirillum; ever since we discovered flagella in S. volutans, there exists between Spirillum and Ophidomonas no distinction at all, if it can be assumed that an organ of locomotion will later be found in the smaller spirilla. We have, therefore, only one choice, either to discard Ophidomonas as a distinct genus and rename the species Spirillum sanguinea - to some extent the mammoth of the bacteria - or conversely to place the naturally equipped flagellated spiral organisms (S. volutans, S. jenensis, and S. sanquinea) under Ophidomonas and retain the name Spirillum exclusively for the smaller species (S. tenue, S. undula), so long as no flagellum is discovered for the smaller ones. If such happens, the name Spirillum would be adandoned.

A portion of Cohn's Plate III (1872), in which drawings of the spiral organisms are shown, is reproduced as Fig. 3. Although Cohn worked only with impure cultures, his observations on the morphology of the bacteria were so acute that his descriptions and illustrations were used by the bacterial taxonomists who followed him in the identification of the species described by him. Cohn's drawings are a decided improvement over those of Müller (1786) and Ehrenberg (1838) in that the morphology of the organisms shown in the drawings is distinctive. In Fig. 3, the drawing of Bacillus ulna could be used for a species of the genus Bacillus today; his spiral organisms are also well drawn.

After the development of the pure culture technique, many systems of classification of the bacteria were published. Insofar as the spiral organisms are concerned, however, many of these systems are unimportant and need not be considered.

BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY

Migula's Taxonomy

Migula published a summary of his first system of classification in 1894 and expanded it in 1895 in Engler and Prantl's Die naturlichen Pflanzenfamilien, Teil 1, Schizophyta. His later work, System der Bakterien (1897, 1900) developed and amplified his ideas on classification. Many of the diagnostic characters used today in the separation of the genera of the bacteria were first proposed by Migula.

Migula, in 1895, clearly distinguishes the several genera of spiral organisms, as shown in the following key:

Migula's Key to the Genera of Spiral Bacteria

A. Cells rigid, non-flexible, spiral.	
a. Cells without organs of locomotion.	1. Spirosoma
b. Cells with organs of locomotion (flagella).	
1. Cells with one, very seldom 2-3 polar flagella.	2. Microspira
2. Cells with polar flagella in tufts.	3. Spirillum
B. Flexible cells.	4. Spirochaeta

Migula adopted the name Microspira, first proposed by Schröter (1886) for the cholera organism, to replace Vibrio. His (1897, p. 23) reasons for nonrecognition of the Vibrio are stated as follows:

> . . . The genus Vibrio had on the whole little good fortune; with every investigator its characteristics changed and the species originally included in the genus were transferred in part to the bacilli and in part to the spirilla.

Migula differentiated the comma or slightly curved organisms from those showing a corkscrew spiral also on the basis of the kind and number of flagella. He placed his spiral organisms either in the genus Microspira (1 - 3 polar flagella) or in the genus Spirillum (tufts of polar flagella). Migula (1900) admitted some difficulty in determining the number of flagella in some species of Spirillum since the

flagella were often agglutinated into bundles resembling a single flagellum. He (1895) noted further that the single flagellum of the species of Microspira usually showed a helical curvature whereas the compound flagellum of Spirillum showed flagella which were seldom curved or at most displayed only a half-curve. Migula's ideas on flagellation are best shown by his illustrations in Die Pflanzenfamilien. A plate of drawings showing flagellation in some species of the bacteria is reproduced as Fig. 4. Differences in flagellation between species of Microspira and Spirillum are shown in the drawings H through M.

Migula (1900, p. 875) confesses to some difficulty in differentiating between species of the genus Pseudomonas and those of the genus Microspira, there being an almost continuous gradient which he described as follows:

> The limits given to Microspira are perhaps artificial ones; it is not always possible to judge whether we are dealing with the single flagellated forms of a Microspira or a Pseudomonas. Slightly curved forms, particularly in stained preparations, can be observed in old Pseudomonas cultures.

Migula (1900) relied on the length of the flagellum (illustrated in drawings C and D, Fig. 4, for species of Pseudomonas) to differentiate between the species of the two genera since he states that species of Microspira never showed a flagellum much longer than the length of the cell, whereas the species of Pseudomonas usually had a flagellum which greatly exceeded the cell length. (This character is not shown in his drawing of Pseudomonas pyocanea (Fig. 4)). He also states that all species of the genus Pseudomonas are fluorescent, whereas species of the genus Microspira are not.

Migula (1895) placed all the flagellated rigid spiral organisms in one genus, combining Ehrenberg's two genera, Spirillum and Ophidomonas, in the genus Spirillum. He separated the colorless and colored forms by the creation of subgenera, placing the colorless forms (including Spirillum rubrum v. Esmarch) in the new subgenus Euspirillum; the colored forms containing cell inclusions of sulfur were placed in the subgenus Thiospirillum Winogradsky.

Migula (1900) recognized a large number of species of the genus Spirillum; many of these have either been

transferred by later authors to other genera or have been regarded as inadequately described. In regard to the number of described species, Migula says:

> The number of existing species of Spirillum is much greater than those described thus far, as pure cultures are as yet only slightly successful and most of those described are probably collective species. Spirillum leucomelaenum Perty may be said to be not one species but to represent certain developmental stages of various species.

Of the species of Spirillum recognized by Migula (1900), the seventh edition of Bergey's Manual (1957) included S. tenue, S. undula, S. volutans, and S. serpens. Migula considered the culture of S. tenue, isolated by Beijerinck (1893), to be a collective species. Migula transferred Cohn's two species of Vibrio to the genus Spirillum. In regard to this change, in the discussion of the genus Vibrio, Migula (1897) says:

> Between Vibrio and Spirillum there is, according to Cohn's diagnosis, no difference, the fact that the undulating curvature is in reality only delicate spirals was suspected by Cohn.

The description of species of Spirillum given by Migula (1900) leaves much to be desired. Although measurements are given for the cells of the species, the dimensions are often rather vague; i.e., in the description of S. rugula, the diameter of the cell is stated to be "twice the thickness of Bacillus subtilis." He also described many species of Spirillum as liquefying gelatin and growing in pour plate cultures; two characters seldom found by recent workers. Two plates of photographs from Migula's System der Bakterien (1897 and 1900), which show the morphology of cultures of Spirillum, are reproduced in Figs. 5 and 6. Although these photographs illustrate the morphology of the organisms observed, apparently they do not illustrate adequately Migula's proposals as to the use of flagella in the differentiation of bacteria as well as do the drawings shown in Fig. 4. Such diagrammatic or composite drawings show general features and are never intended to be taken as illustrating an individual organism. Those who have hunted through preparation after preparation in search of the "typical cell" will attest to the value of such diagrammatic drawings.

PLATE I.

Figure 1

Table VI. O.F. Muller's Animacula infusoria fluviatilia et marina, 1786. (Courtesy, Cornell University Library.)

1. Vibrio lineola
2. Vibrio ruqula
3. Vibrio bacillus
4. Vibrio undula, small mass of cells emerging from a larger mass.
5. Vibrio undula, a mass collected around a filament of one of the Confervae.
6. Vibrio undula, greatly magnified. d. at rest e. swimming
7. Vibrio serpens, magnified.
8. Vibrio serpens, highly magnified. a. intestine
9. Vibrio spirillum

Figure 2

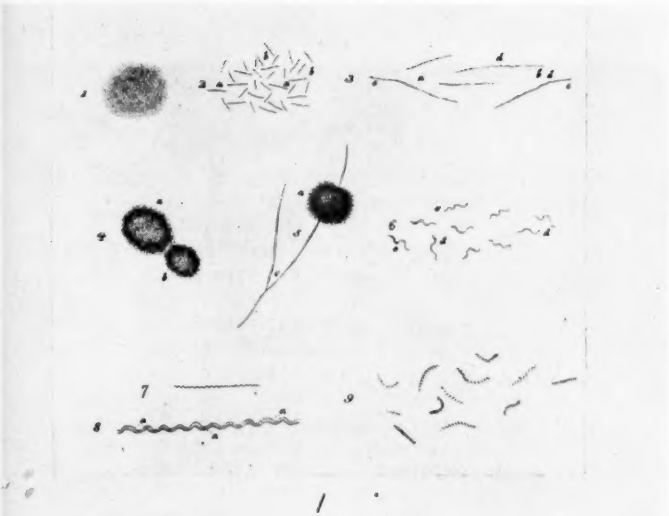
Table V from C.G. Ehrenberg's Die Infusionthierchen als volkemmene Organismen, 1838. (Courtesy, Library of Congress.)

I. Bacterium trioculare; II. Bacterium enchelys; III. Bacterium punctum; IV. Bacterium lineola; V. Vibrio tremulans; VI. Vibrio bacillus; X. Spirochaeta plicatilis;

XI. Spirillum tenue; 25 spiral filaments differing in length of the spiral curves, often exhibiting a vibrating motility; original 300 X.

XII. Spirillum undula; with two magnifications.
1. Group of 18 monad-filaments magnified 300 X.
2. 12 different types of spiral curvature, magnified 800 X.
3. Indicates the line of motion of single spiral rods.

XIII. Spirillum volutans;
1. Organism observed in St. Petersburg. 6 spiral rods, with fewer spiral windings 1/96 as many.
2. Magnification of 800 X, with 10 filaments in different degrees of aggregation, from single organisms in fission to coils.
3. In a plant infusion and is as long as 1/48 of a line (1 line = 1/12 inch or 2.2 mm) magnified 800 X.



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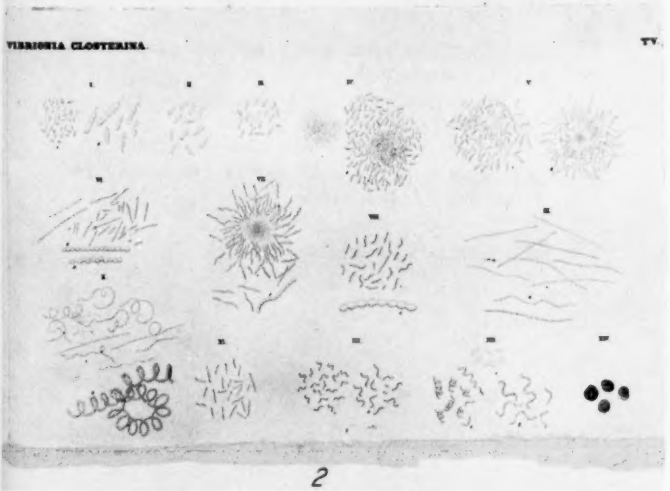

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PLATE II.

Figure 3

F. Cohn's Table III, Beitrage zur Biol. d. Pflanzen I. (Heft 1), 1872. (Magnification 650 X)

15. Bacillus ulna, single filament and longer rod, with several filaments breaking in division.

16. Vibrio ruqula, single cells or those in division with one showing an apparent swelling in consequence of the quick rotation.

17. Vibrio serpens, long or short rods, dividing into smaller rods; two of the rods twisted about each other.

18. A cluster of V. serpens with the cells matted together.

19. Spirillum tenue, single cells and clusters matted together.

20. Spirillum undula.

21. Spirillum volutans with two of the spiral rods wound around each other.

22. Spirochaeta plicatilis.

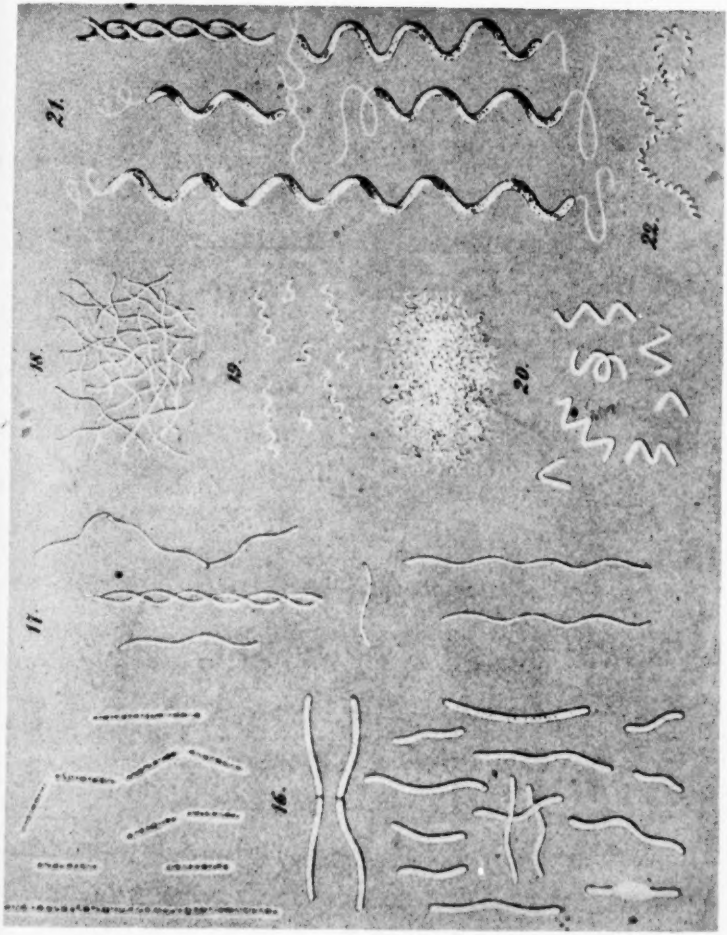

21.
22.
18.
19.
20.
11.
16.

PLATE III.

Figure 4

W. Migula's figure 1 from Schizomycetes, Teil 1, in Engler and and Prantl's Die naturlichen Pflanzenfamilien, 1895.

A. Bacillus subtilis and Spirillum undula, cell wall which has been separated from the plasma body with the flagella attached.

B. Planococcus citreus (Menge) Migula.

C. Pseudomonas pyocyanea (Gessard) Migula.

D. Pseudomonas macroselmis Migula.

E. Pseudomonas syncyanea (Ehrenberg) Migula.

F. Bacillus typhi Gaffky.

G. Bacillus vulgaris (Hauser) Migula. Filament and single cell.

H. Microspira comma (Koch) Schroter.

J. Spirillum rubrum v. Esmarch, short cells.

K. Spirillum rubrum v. Esmarch, long cell.

L. Spirillum undula (Müller) Ehrenberg.

M. Spirillum undula (Müller) Ehrenberg. The flagella are agglutinated into a bundle.

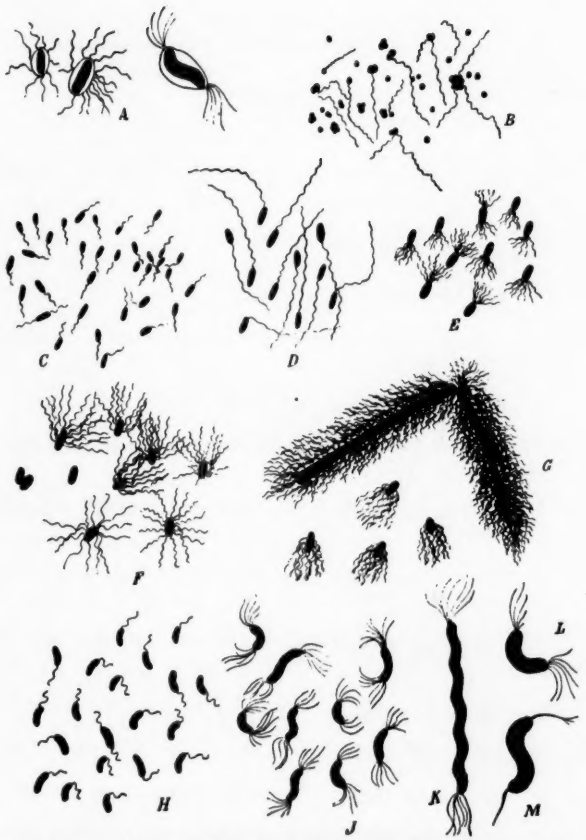


Fig. 1. *A Bacillus subtilis* Cohn und *Spirillum Undula* Ehrenb., Membran mit daran hängenden Geißeln, vom Plasmakörper abgehoben. — *B Planococcus citreus* (Mongo) Migula. — *C Pseudomonas syncyanea* (Gessard) Migula. — *D P. macrostoma* Migula. — *E P. syncyanea* (Ehrenb.) Migula. — *F Bacillus typhi* Gaffky. — *G B. vulgaris* (Hauser) Migula, Fäden und einzelne Zellen. — *H Microspira Comma* (Koch) Schröter. — *J Spirillum rubrum* v. Esmarch, kurze Zellen. — *K S. rubrum* v. Esmarch, lange Zelle. — *L S. undula* (Müller) Ehrenb. — *M S. undula* (Müller) Ehrenb., Geißeln zu einem Strang verklebt. — Sämtliche Abbildungen nach mit der Löffler'schen Beize behandelten Deckglastrockenpräparaten, 1000/1. (Original.)

PLATE IV

Figure 5

Table III from V. Migula's System der Bakterien, Volume I, 1897.

3. Microspira nigricans (Weibel) Migula. Flagella stain.

4. Spirillum serpens (Müller) Winter. Flagella stain.

5. Spirillum undula (Ehrenberg). Flagella stain. Flagella agglutinated into unequal bundles.

6. Spirillum undula (Ehrenberg). Flagella stain. Flagella agglutinated in a single thick bundle.

7. Thiospirillum rufum (Perty) Winogradsky. Flagella stain.

8. Spirillum rubrum v. Esmarch. Flagella stain. By repeating the mordant and stain, very thick flagella are obtained.

(All flagella stains by Löffler's method. Original 1000 X)

Figure 6

Reproduction of Table XVII from W. Migula's System der Bakterien, Volume II, 1900.

1. Microspira albis. Flagella stain after Löffler. 1000 X.

2. Microspira tyrogena. Ramifications. Old broth culture. 1000 X.

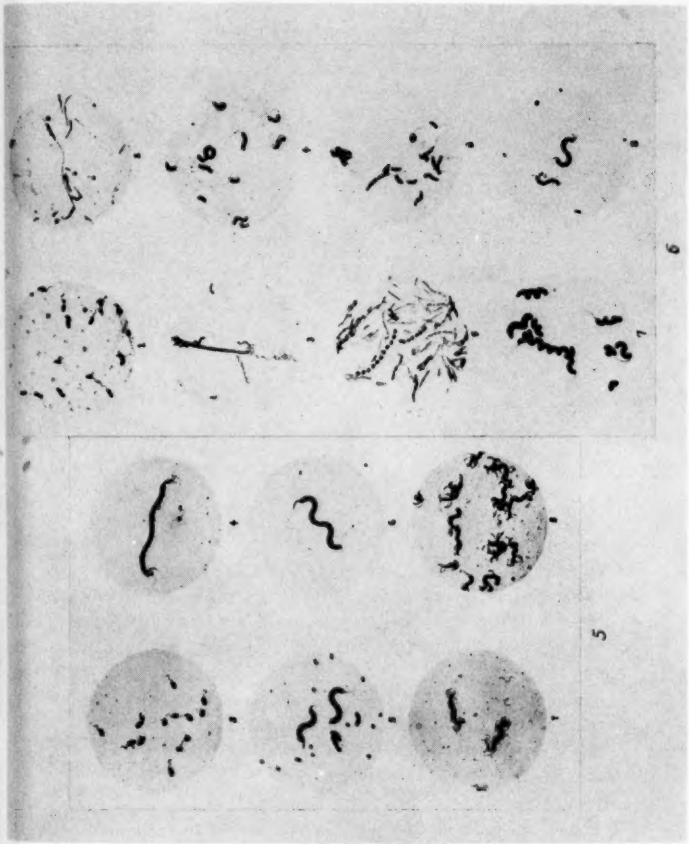
3. Spirillum rubrum. Long spirals twisted together. Broth culture. 1000 X.

4. Spirillum rubrum. Flagella stain after Löffler.

5. Spirillum sporiferum. From a decaying bean infusion.

6. Spirillum tenue. Flagella stain after Löffler.

7. Spirillum kutscheri. Flagella stain after Löffler.

8. Spirillum undula. Flagella stain after Löffler. From a straw infusion.

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